and theoretical perspectives. A recent modification proved successful in reducing the number of Trp anchors to one near each end of the peptide. The resulting GWALP23 (acetyl-GGALW^5(LA)$_6$LW^{19}LAGA-[ethanol]amide) displays greater sensitivity to lipid-peptide hydrophobic mismatch. Now we further modify GWALP23 to incorporate a single tyrosine, replacing W^5 with Y^5. The resulting peptide, Y^{5}GWALP23 (acetyl-GGALY^5(-LA)$_6$LW^{19}LAGA-amide) has now only a single remaining Trp residue which is accessible for fluorescence experiments. By labeling specific alanines in Y^{5}GWALP23 with deuterium, we were able to use solid-state ^{2}H-NMR spectroscopy to examine the peptide orientation in hydrated lipid bilayer membranes. The peptide orients well in membranes. The substitution of Y^5 for W^5 has remarkably little influence upon the GWALP23 apparent tilt or dynamics in bilayer membranes of DOPC, DMPC or DLPC. A second analogue with double Tyr anchors, Y^{4,5}GWALP23, was found to be generally less responsive to the bilayer thickness and to exhibit lower apparent tilt angles and possibly more extensive dynamics. Indeed the case with multiple Tyr anchors seems to be quite similar to the situation when multiple Trp anchors are present, as in the original WALP model peptides.

3442-Pos Board B547
Comparison of Proline Substitutions at Positions 8 and 10 in WALP19
Joseph M. Courtney, Vitaly V. Vostrikov, James F. Hinton, Roger E. Koeppe II.
Proline is often found in the transmembrane helices of naturally occurring membrane-spanning proteins. It is thought that proline introduces a kink and disrupts the helical structure, possibly causing an overall loosening of the helix, forming a "molecular hinge." The influence of proline at the center of the model peptide WALP19-P10 (acetyl-GWWLALALAP^{10}ALALALWWA-ethanolamide) has been characterized in several lipid bilayer membranes (see Biochemistry 48, 11883). In this research, we employ solution NMR spectroscopy to examine the structures of WALP19-P8 (acetyl-GWWLALAP^{8}ALALALALWWA-ethanolamide) as well as WALP19-P10 in SDS micelles. The spectral quality is better for WALP19-P8 than for the proline-10 peptide. Based upon >135 restricting NOE assignments throughout the sequence (including interactions that link residues L6 and A9, as well as A7 and L10, on either side of the proline), we have determined a kinked structure for WALP19-P8. Preliminary analysis suggests that some of the intrahelix hydrogen bonding is disrupted both near and far from the proline, indeed resulting in a general loosening of the helical structure. The spectra for WALP19-P10 are more difficult to interpret structurally, despite a high number of assigned NOE restraints (>220). Local dynamics near the hinge region and sparse NOE connectivity in the vicinity of proline permit multiple solutions for the WALP19-P10 backbone geometry. Conversely, local bond dynamics appear to be a minor factor in WALP19-P8. The interplay among proline location, spectral quality, and structure determination will be discussed.

3443-Pos Board B548
Influence of Glycine Substitutions on Designed Proline-Containing Transmembrane Peptides
Christopher D. DuVall, Vitaly V. Vostrikov, Denise V. Greathouse, Roger E. Koeppe II.
Membrane-spanning WALP peptides of general sequence acetyl-GWW(LA)$_n$LWWA-amide, in which ''n'' may range from about 3 to 12, have been well characterized. A kink induced by a central proline residue in WALP19-P10 (acetyl-GWWLALALAP^{10}ALALALWWA-ethanolamide) has also been characterized (see Biochemistry 48: 11883). It was furthermore observed that the ^{2}H-quadrupolar splittings from labeled alanine side chains in WALP19-P10 were especially sensitive to Leu-to-Ala substitutions elsewhere in the sequence. In this work, we examined the consequences of Leu-to-Gly substitutions within the proline-containing WALP19-P10, from a perspective that Leu-to-Gly substitutions could reasonably be expected to produce larger consequences than Leu-to-Ala substitutions. Circular dichroism spectra indicate small reductions in helicity for Gly substitutions in WALP19-P10, with the mean residue ellipticity values being similar for Leu-to-Gly substitutions on either side of the proline. Using ^{2}H-alanines as probes within the sequence, we have found from deuterium NMR spectroscopy of peptides incorporated within oriented lipid bilayer samples that the segment N-terminal to proline is generally more susceptible to Leu-to-Gly substitutions on both sides of the proline. In contrast, the C-terminal domain is largely unaffected by the glycine substitutions in either the N- or C-terminal segment. Substitutions near the C-terminus have little effect on either segment.

3444-Pos Board B549
Helix-Helix Interactions in Phospholipid Model Membranes as a Function of Acyl Chain Unsaturation
Bo Wang, Scott E. Feller.
Previous work in our laboratory has identified lipid-protein interactions that appear unique to the class of lipids having polyunsaturated fatty acids (PUFA). In this work we extend these studies to examine the effect of PUFA on lipid mediated protein-protein interactions. Specifically, we have used molecular dynamics (MD) computer simulation methods to compute the potential of mean force (PMF) between a pair of alpha helical peptides, i.e. the free energy as a function of separation. Constant pressure, constant temperature simulations of the identical peptides in phosphidylcholine bilayers with saturated chains (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and those containing PUFA (1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine) have been carried out using the techniques of umbrella sampling and the weighted histogram analysis method. These lipids were carefully chosen for their near identical hydrophobic thicknesses so that the effect of unsaturation could be uniquely examined.

3445-Pos Board B550
RIP Pulchellin Isoforms: Biomembrane Models Showing Different Intoxication Mechanisms Between Them
Thatyane Morimoto Nobre, Ana Paula Ulian Araujo, Rosangela Itri, **Leila M. Beltramini**.
Pulchellin, a type-2 **R**ibosome **I**nactivating **P**rotein (RIP), whose action mechanism proposed would be initiate by B-chain binding on glycoconjugate onto the cell surface (lectinic chain). Into the cell, the A-chain (toxic chain) binding in ER lipids and stopping protein synthesis at ribosome inducing cell death. Was showed that Puchelin isoforms have different toxicity and were separated subgroups PI, PII, PIII, PIV based: toxicity, secondary structure, specificity for sugar binding. PII and PIV subgroups present structural identity above 70%, however PII is five-fold more toxic than PIV (Castilho, et al. FEBS journal, 2008). The present studies show results of interaction between model membranes and the two isoforms (PII and PIV) using Langmuir monolayers (LM) and giant unilamellar vesicles (GUVs). PII and PIV bond to LM-DPPC in the same extension, but when monolayers contented 5% of GalCer, PII remained strongly interacting with the mixed monolayer and PIV seems to be inhibited by the presence of the glycolipids. In GUVs-POPC, both PII and PIV destroyed the vesicles, however, in GUVs-POPC+5%GalCer, only PII induced vesicles decreased in their surface around 30% and some invaginations could be observed. After 20 minutes, small vesicles were observed inside of the GUV structure. For PIV, instead of invaginations, only fluctuations on the GUV surface were verified. Increasing PIV concentration the GUVs were destruction. In view of the results, one can conclude that the key for understanding differences in pulchellin isoforms toxicity is the interaction between B-chain lectinic and the glycosyl residues on cell surface.
Supported by Brazilian Agencies: FAPESP, CNPq

3446-Pos Board B551
A Putative Role for Lipid-Protein Interactions in the Localisation of Glycosyltransferases within the Cell?
Phedra Marius, Daniel A. Holdbrook, Syma Khalid, **Philip T.F. Williamson**.
Fukutin-I is a member of a family of putative O-linked glycosyltransferases involved in the glycosylation of the dystrophin complex. Mutations in this family of proteins have been linked to number of congenital muscular dystrophies and are characterised by the hypoglycosylation of the alpha-dystroglycan. The function of these proteins relies critically on their location within the endoplasmic reticulum (ER)/Golgi Apparatus (GA). The retention of proteins in these compartments is thought to be mediated by the interaction of the N-terminal transmembrane domain with the atypical lipid composition of these compartments although the molecular basis for this retention is far from clear. To address this question, we are currently studying how the composition of the lipid bilayer within the ER/GA modifies the structure, oligomeric state and lateral segregation of these proteins. Preliminary studies have established an expression system for the production and labelling of the transmembrane domains of these proteins. Using a combination of liquid and solid-state NMR we are characterising how the structure of the N-terminal transmembrane domain of Fukutin-I varies as a function of lipid/detergent composition. Solid-state NMR methods are revealing how the lipid bilayer composition changes the dynamics of the protein within the bilayer thus providing insights into the proteins oligomeric state. In conjunction with cross-linking and fluorescence resonance

energy transfer measurements these studies are revealing significant differences in the oligomeric state of the N-terminal transmembrane domain of Fukutin-I in response to bilayer composition with implications for the regulation of protein trafficking.

3447-Pos Board B552

The Intermembrane Ceramide Transport Catalyzed by CERT is Sensitive to the Lipid Environment

Peter Mattjus, Jessica Tuuf, Matti Kjellberg, Julian G. Molotkovsky, Kentaro Hanada.

The in vitro activity of the ceramide transporter, CERT has been studied using a fluorescence assay. CERT is responsible for the in vivo non-vesicular trafficking of ceramide between the endoplasmic reticulum and Golgi. In this study we have examined how the membrane environment surrounding the ceramide substrate, the membrane packing density and the membrane charge, are affecting the ceramide transfer activity. To examine this we have used an anthrylvinyl-labeled ceramide analogue. We found that if ceramide is in a tightly packed environment such as in sphingomyelin or dipalmitoylphosphatidylcholine containing membranes, the CERT transfer activity is markedly reduced. Ceramide in fluid membranes on the other hand are available for CERT mediated transfer. CERT also favors membranes that contain phosphatidylinositol 4-monophospate, due to its binding capacity of the pleckstrin homology domain towards phosphatidylinositol 4-monophospate. From this study we conclude that the membrane matrix surrounding ceramide, that is ceramide miscibility, is largely affecting the transfer activity of CERT.

3448-Pos Board B553

Membrane Thickness Dependence of Non-Mammalian Prestins

Chisako Izumi, Jonathan E. Bird, **Kuni H. Iwasa**.

Prestin is significant for voltage-dependent somatic motility of outer hair cells in the cochlea, which is important for mammalian hearing. This membrane protein undergoes conformational changes in response to changes in the membrane potential in a manner similar to piezoelectricity. Previously we have shown that mammalian prestin is sensitive to membrane thickness by changing membrane thickness by perfusion of gamma-cyclodextrin loaded with phosphatidylcholines with various hydrocarbon chain lengths. A reduction in membrane thickness led to a positive shift in the operating point of the membrane potential at which charge transfer associated with motile response takes place. The shift observed was up to 150 mV. An increase in membrane thickness had the opposite effect. Shifts were about +6 mV for 1% reduction in thickness. This result was interpreted as an indication that conformational change of prestin, namely the conformation with larger membrane area has thinner hydrophobic area that interfaces lipid bilayer. In the present study, we examined whether or not non-mammalian prestins are also sensitive to membrane thickness. We found that the membrane thickness dependence of platypus prestin was quantitatively similar to that of mammalian prestin. In contrast, chicken prestin did not show a systematic membrane thickness dependence. These results demonstrate that chicken prestin does not undergo conformational changes that are similar to those of mammalian or platypus prestin. These findings are therefore consistent with the presence of mechano-electric coupling in platypus prestin and the absence of such coupling in chicken prestin.

3449-Pos Board B554

A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor

Joost Ballering, Larisa E. Cybulski, Diego de Mendoza, J. Antoinette Killian.

The *Bacillus subtilis* membrane harbors the temperature sensing and signaling protein DesK. At low temperatures it triggers expression of a desaturase, which introduces double bonds into pre-existing phospholipids, thereby regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called 'minimal sensor'. This simple system offers excellent perspectives to study the molecular detail of a biologically very important mechanism. As a first step here we analyzed membranes of *Bacillus subtilis* grown at different temperatures with several biophysical techniques including ^{31}P-NMR and Differential Scanning Calorimetry. We analyzed the membrane lipid headgroup and acyl chain composition and we identified transition temperature fluctuations related to the growth temperature. We found significant differences in membrane lipid composition and phase behavior for *Bacillus subtilis* membranes depending on growth temperatures. The next step is to synthesize the transmembrane segment of the minimal sensor and incorporate it in these membranes exploiting their properties to elucidate the molecular mechanism of thermosensing in *Bacillus subtilis.*

[1] Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. Membrane thickness cue for cold sensing in a bacterium. *Curr Biol*. 2010 20 (17):1539-44

3450-Pos Board B555

Thermosensor Desk Measures Membrane Thickness

Larisa E. Cybulski, Mariana Martin, Diego de Mendoza.

The bacterium *Bacillus subtilis* adjusts the composition of membrane lipids to cope with temperature variations. The histidine kinase DesK is a five-pass transmembrane thermosensor suited to remodel membrane fluidity in *B. subtilis* according to temperature. To understand the mechanism of sensing, individual transmembrane segments (TMS) were fused to the cytoplasmic catalytic domain of DesK (DesKC) and the ability to respond to temperature analyzed. Surprisingly, a hybrid TMS composed of 17 aminoacids of the first TMS and the C-terminal 14-residue portion of TM5 fused to DesKC, fully retains *in vivo* and *in vitro* the sensing properties of full-length DesK [1]. Besides, when chimerical fusions of DesKC to either TMS1 or TMS5 are expressed *in vivo* individually in a desK- strain, thermosensing is lost. Nevertheless, when they are co-expressed thermosensing is recovered; suggesting that interactions between TMS1 and TMS5 are needed for signaling.

The N-terminus of TMS1 contains three hydrophilic aminoacids near the lipid-water interface creating an instability hot spot. We showed that this boundary-sensitive motif controls the sensing and transmission activity. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophilic spot. This hypothesis is supported through biochemical studies including *in vitro* reconstitution of the MS in liposomes with different chain length.

[1] Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. Membrane thickness cue for cold sensing in a bacterium. *Curr Biol*. 20: 1539-1544, 2010

3451-Pos Board B556

Modulation of the Activity of an Integral Membrane Protein by Phospholipids in Mixed Micelles

Martin M. Dodes Traian, Diego I. Cattoni, Valeria Levi, F. Luis González-Flecha.

Although the importance of lipid-protein interactions in determining the biological function of integral membrane proteins is well recognized, the underlying molecular mechanisms remain unclear. In this work we explore the modulation of the enzymatic activity of an integral membrane protein by phospholipids, using the Plasma Membrane Calcium Pump (PMCA) reconstituted in mixed detergent $C_{12}E_{10}$-phospholipid micelles as model system. Increasing amounts of phospholipids were added to purified PMCA preparations reconstituted in detergent micelles producing a reversible increase in activity reaching a maximum value. No major structural changes occur during this process neither in the protein as assessed by far UV circular dichroism (CD) and tryptophan fluorescence nor in the micellar system as determined by fluorescence spectroscopy and fluorescence correlation spectroscopy (FCS). In addition the relative affinities of phospholipids for the PMCA transmembrane region were evaluated by a FRET method using a pyrene labelled PC as fluorescent probe. These results were analyzed in terms of a macroscopic model that includes the affinities of the phospholipids covering the PMCA transmembrane region and a transduction parameter that correlates the composition of the boundary monolayer with the enzyme activity. The model predictions show good agreement with the experimental data, linking amphiphile/protein interactions with enzymatic activity.

3452-Pos Board B557

Small-Angle Neutron Scattering Reveals Colicin N Inserts into Clefts on the Outside of the OmpF Trimer

Christopher L. Johnson, Luke A. Clifton, Alexandra Solovyova, Phil Callow, Kevin L. Weiss, Helen Ridley, Anton P. le Brun, Stephen A. Holt, Jeremy H. Lakey.

We wish to understand the mechanism by which colicins translocate across the outer-membrane of competing bacteria to mediate cell death. Pore-forming colicin N hijacks *E. coli* outer-membrane protein OmpF and exploits it as both a receptor and translocator to cross the outer-membrane [1]. It is currently